IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.:	10/802,240
Applicant:	Paolo Cavassini, Paolo Cicognani
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DECLARATION OF EMILIO CERCHIARI

1. I presently reside at Nonantola (MO), Italy.

2. I am a consultant to Valentini S.R.L., the current assignee of the above-referenced U.S. Patent Application Serial No. 10/802,240, filed March 17, 2004. Valentini S.R.L. pays me for the consulting services I perform for them.

3. My educational background is as follows. I received a degree in Animal Science from the University of Bologna, in Italy, in 1978. From 1978 to 1980 I was an employee of Christian Hansen Lab., Copenhagen, Denmark, working in the position of animal nutritionist. From 1980 to 1982 I was an employee of an Italian company, AGROLABO, that was the distributor for ALBION USA in Italy, distributing nutritional products. From 1983 to the present I have been an independent consultant to companies in Europe and the USA in the area of animal nutrition.

4. Certain tests were conducted by me or under my supervision with respect to the invention described in the above-referenced U.S. Pat. App. No. 10/802,240. The tests were conducted to demonstrate the criticality, in the present invention, of using a coating made up of two distinct layers of different materials, with each layer having a different functionality; i.e. the inner layer to provide ruminal by-pass protection and the outer layer to provide resistance to the harsh characteristics of the pelletization process. The tests were conducted to demonstrate that it was critical to have these two different, distinct layers in order to provide effective protection against ruminal degradation. The tests are described as follows.

Materials and methods

Granules were provided which had a core containing choline chloride and a binder comprising zinc stearate, magnesium stearate and calcium stearate (total amount of binder 7%). The granules were then coated with different coatings (as described below) and tested.

Choline chloride was chosen as the active ingredient since it is one of the most hygroscopic of the usual ingredients and, therefore, one of the most sensitive to ruminal degradation among the active ingredients that are normally administered to ruminants.

The granule cores containing choline chloride were coated with the following different layers:

1. Core coated with a single layer essentially made of carnauba wax having a melting point of 80-100°C; this product was labelled as: **MLC 2°** granule.

2. Core coated with a single layer made of a 1:1 (by weight) mixture of (a) hydrogenated palm oil and (b) carnauba wax having a melting point of 80-100°C; this product was labelled as: **Mix** granule.

3. Core coated with a single layer essentially made of lipids (hydrogenated palm oil); this product was labelled as: **MLC 1°** granule.

4. Core coated with two layers. The inner layer is essentially made of hydrogenated palm oil and the outer layer is essentially made of carnauba wax having a melting point of 80-100°C; this product was labelled as: **MLC** granule.

5. Preparation of a starch-based (corn starch and wheat starch) animal feed(*) containing 10% of **MLC 2°** granules *(item 1)*. This feed was subjected to a pelletization process at a temperature of about 77-80 °C. The final product was labelled as: **MLC 2°** pellet.

6. Preparation of a starch-based (corn starch and wheat starch) animal feed(*) containing 10% of **Mix** granules *(item 2)*. This feed was subjected to a pelletization process at a temperature of about 77-80 °C. The final product was labelled as: **Mix pellet**.

7. Preparation of a starch-based (corn starch and wheat starch) animal feed(*) containing 10% of **MLC 1°** granules *(item 3).* This feed was subjected to a pelletization process at a temperature of about 77-80 °C. The final product was labelled as: **MLC 1° pellet**.

8. Preparation of a starch-based (corn starch and wheat starch) animal feed(*) containing 10% of **MLC** granules *(item 4).* This feed was subjected to a pelletization process at a temperature of about 77-80 °C. The final product was labelled as: **MLC pellet**.

For items 5 - 8, the temperature measured at the end of the pelletization process was 78.5 °C.

(*) The choice of corn and wheat starches as base ingredients for the animal feed was due to the fact that they do not contain choline. Therefore, the feed ingredients would not interfere with the analysis.

The four different granules and the four different pellets obtained as above were subjected to a ruminal resistance test *in vitro*, as follows.

For the tests there was employed an *in vitro* fermenter Daisy II 200 (Ankom Technology, Fairport, NY, USA). This fermenter allowed the simulation, in a closed environment, of the fermentation process of the rumen of a ruminant.

The *in vitro* methodology comprised the step of incubating a porous bag (5 x 5 cm; porosity: 25 µm) filled with the item (granule or pellet) to be tested (250 mg of dry sample, finely ground) in a glass vessel containing ruminal liquid (400 ml) diluted in a mineral solution (1600 ml). The vessel was introduced into the "Daisy" fermenter and kept at a temperature of 39°C under continuous stirring for 12 hours. At the end of the incubation the bag was rinsed first with water and then with neutral detergent solution, in order to remove the microbial residues and possible soluble particles left inside. The bag was placed in a ventilated oven at 60°C for one night; afterwards, the percentage of rumen resistance was determined.

Results and discussion

The results of the ruminal degradability tests of the samples are listed in Table 1.

Table 1

Item Tested:	**Ruminal degradation resistance (by-pass) [1]** ***(average of three analytical measurements) 12 hours of incubation in Daisy® Ankom***
MLC 2° granules	**28.15%**
MIX granules	**35.10%**
MLC 1° granules	**86.50%**
MLC granules	**87.80%**
MLC 2° *PELLET*	**21.00%**
MIX *PELLET*	**16.40%**
MLC 1° *PELLET*	**22.86%**
MLC *PELLET*	**83.55%**

[1] *The analysis was made with an enzymatic method (choline-oxidase) with titration of choline chloride.*

5. The test results of Table 1, above, demonstrate that it is critical to have 2 separate and distinct layers, the first or inner layer being a lipid layer to provide effective rumen protection (by-pass) and a second or outer layer being a hard wax layer to provide protection from the harsh pelletization process. This can be seen from the last four items of Table 1, being the four different pellets. The pellet having just a single layer of hard wax (MLC 2° Pellet) showed poor resistance (21.00%). The pellet having a single layer coating made of a mixture of hard wax and lipids (MIX Pellet) also had poor resistance (16.40%). The pellet having only a single layer of lipid (MLC 1° Pellet) also had poor resistance (22.86%). Only the pellet having two separate layers, the inner layer being lipid coating and the outer layer being hard wax coating (MLC Pellet) had excellent resistance (83.55%). In summary, all three of the single layer pellets

(single layer of hard wax, single layer of a mixture of hard wax and lipid, and single layer of lipid), all showed poor rumen resistance (by-pass). Only the double layer pellet (MLC pellet) having an inner layer of lipid and an outer layer of hard wax, showed good rumen resistance.

6. The tests of the four granules (the first four items tested in Table 1) show that, for granules that have not been pelletized, a granule having a layer of lipid next to the core will provide effective ruminal resistance. The MLC 1° granule has a single layer of lipid and showed 86.50% resistance and the MLC granule (inner layer of lipid and outer layer of hard wax) also showed good resistance (87.80%). The MLC 2° granule has only a single layer of hard wax and showed poor resistance (28.15%); the MIX granule has a 1:1 by weight mixture of lipid and hard wax but showed poor resistance (35.10%). It is important to note that when the granule with a single layer of lipid (MLC 1°) is tested as a granule, it has good resistance (86.50%), but when it is pelletized without a protective second layer of hard wax, the performance is very poor (MLC 1° pellet has resistance of 22.86%). However, when the two layer (MLC) granule is pelletized, very little resistance is lost (from 87.80% for MLC granule to 83.55% for MLC pellet). In summary, it is critical to have two different layers, the first or inner layer being a lipid layer and a second or outer layer being a hard wax layer, in order to provide a pellet having effective rumen protection. None of the single layer granules (single layer lipid, single layer hard wax or single layer mixture of lipid and hard wax) can provide effective rumen protection when put into pellet form.

7. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the patent application or any patent issued thereon.

July 15, 2010

Date

EMILIO CERCHIARI